On the Nematodes of the Common Earthworm.

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With Plate 37 and 2 Text-figures.

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INTRODUCTION.

(1) Survey of the Literature.

It has long been a matter of common knowledge that larval nematodes inhabit the common earthworm, Lumbricus terrestris, Linn. They are found both in the cœlom and in the nephridia. Those occurring in the cœlom are enclosed in cysts or capsules, by which movement is restricted or entirely prevented. Those inhabiting the nephridia, on the other hand, are free and active. The encysted, cœlomic form has long been known as Rhabditis pellio, having been named by Schneider (**1**) as early as 1866. The nephridial form is generally believed to be the same species, but it was not mentioned by Schneider, and I have not been able to find that its identity has been determined by any subsequent investigator, as the following brief survey of the literature will show.

In 1845 Dujardin, according to Bastian (**2**), recorded the existence of nematodes in the general body-cavity of the earthworm. These he placed in the genus Rhabditis. He found that they developed in prodigious numbers, forming whitish masses in the vessels in which he had kept earthworms with moss and damp soil. Bastian says that Dujardin also described a nematode, Dicelis filaria, occurring in great abundance in the nephridia of the earthworm.

In 1858 Lieberkühn, according to von Linstow (**3**), showed that the "Filariæ of the earthworm," after the death of their host, creep out of the cysts, moult, and in a few days develop into mature worms.

In 1864 and 1865 Lankester (**4, 5**) mentioned the nematodes as occurring in the lobes of the seminal vesicles, in the posterior end of the cœlom and imbedded in the muscular layer of the body-wall.

Hitherto only the larvæ had been investigated, but in 1866 Anton Schneider (**1**) described the adults. He stated that they occur "in damp earth and putrefying substances." These terms he must have used to signify dead earthworms decaying in moist earth, for he says—"The larvæ occur encysted in the body cavity of earthworms, especially on the septa," and adds that, as Lieberkühn was the first to point out, they become sexually mature on the decay of the worms. Schneider named the species Pelodera pellio.

In 1873 Bütschli (**6**) described the adult males and females obtained from decaying earthworms in greater detail and with good figures. He noticed certain structural differences between his form and Schneider's but regarded these as unimportant. He altered Schneider's name Pelodera pellio to Rhabditis pellio, Rhabditis being Dujardin's name for the larvæ which he found in the earthworm.

Von Linstow (**3**) in 1882 described the larvæ as well as the adult males and females, and, using a decaying worm, actually developed the encysted larvæ into the sexually mature Rh. pellio.

Railliet (**7**) in 1893 merely quoted Schneider's description.

Keng (**8**) in 1895 watched the encystment of the cœlomic form, but he does not show that he was aware of the identity of the nematodes with which he was dealing.

In 1897 von Erlanger (**9**), working on the segmentation of the egg, made a pure culture of Rh. pellio from strips of the body-wall of an earthworm placed on soil which had been first sterilised and then moistened. He obtained large numbers in this way.

Maupas (**10**) in 1899 made extensive cultures of Rh. pellio with an artificial food-medium. He probably used for parents the encysted larvæ from the freshly killed worm or the mature adults from decaying worms, for, while he speaks about the encysted, cœlomic form, strangely enough he does not mention the active, nephridial larvæ.

In 1900 de Ribaucourt (**11**) described the excystation of the encysted larva. He mentions the nephridial form and explains its probable method of entry. He did not attempt to determine its identity or show that he knew that of the encysted form, but he evidently regarded them as belonging to the same species.

Shipley (**12**) in 1902 summarised the work of the earlier writers. Like them he says nothing of the nematode in the nephridia.

K. C. Schneider (**13**) in 1902 mentioned the encysted larvæ of Rh. pellio occurring in the cœlom. He also says that nematodes, which, according to Anton Schneider, belong to Rh. pellio, are frequent in the lumen of the bladder of the nephridium. But this statement is incorrect, for, as has been seen, Anton Schneider did not mention the nephridial form.

It will be seen from the foregoing survey that, while almost every writer describes the larva of Rh. pellio as living encysted in the cœlom, there are only three who mention the nephridial larva. Dujardin called it Dicelis filaria, evidently regarding it as a different species from the cœlomic Rhabditis form. De Ribaucourt mentions both forms, and though unconcerned with their identity, writes as if he believed them to be the same species. Lastly K. C. Schneider wrongly supposes that Anton Schneider identified the nephridial form as Rh. pellio, whereas he did not even mention it.[1]

Thus although the nephridial form is generally supposed to be the same species as the cœlomic form, Rhabditis pellio, it appears that in reality its identity has never been determined.

(2) Nature of the Research.

The present research, then, was undertaken with the object of identifying, and following out, if possible, the life-history of the active larval nematode inhabiting the nephridia, and in the hope of being able, in so doing, to throw some light upon the relations of sex in the group. The work has proved exceedingly difficult, and the conclusions reached are in some cases largely hypothetical. But the subject is one of great interest, and further work should yield valuable results.

The work has been carried out in the Zoological Laboratory of the University of Birmingham, under the supervision of Professor F. W. Gamble, F.R.S., to whom I am very grateful for his unfailing assistance, by suggestion and by criticism, throughout the course of the investigation. I am deeply indebted to the Board of Agriculture and Fisheries for the award of a research scholarship in Agricultural Zoology, with the aid of which the latter part of the research has been

[1] For a reference to Örley's work, see footnote, p. 622.

carried on. I have also been assisted by a grant which was made me at the commencement of the work from the Endowment of Research Fund of the Birmingham Natural History and Philosophical Society.

Occurrence.

(1) The Encysted Larva inhabiting the Cœlom.

The encysted form is most plentiful in the posterior end of the cœlom. In dissecting Lumb. terrestris a number of flattened and roughly oval bodies of brown matter varying in length from 1 to 5 mm. are to be found lying round the intestine close to the anus, in the compartments formed by the septa. These brown bodies, when placed in water and examined under the microscope, are at first too solid and opaque to show of what they consist. But in some instances a few nematodes are to be seen partly imbedded in them and partly free, their free ends waving about in the water. Occasionally large, white, rounded bodies are present, projecting from the surface. These are cysts of Monocystis.

When one of the brown bodies is placed in water a number of unencysted larval nematodes make their escape from it before very long. In the course of one or two days it gradually disintegrates. It is then seen to be constituted of numerous small, and occasional large, cysts of species of Monocystis, discarded setæ of the worm (sometimes still encased in the setigerous sac), encysted nematodes (of which some have already escaped from their cysts and others are in the act of doing so), and lastly a large quantity of loose brown cellular matter, which consists of broken-down, discoloured amœbocytes, and has held the whole mass firmly cemented together. Lankester (**5**, p. 104), de Ribaucourt (**11**) and K. C. Schneider (**13**, p. 425) have all described these inclusions of the cœlomic fluid at the posterior end of the body, and my observations agree with theirs.

The cyst enclosing the nematode fits round its body closely, but, being slightly longer than the nematode, allows it to move a short distance backwards and forwards. It appears to be formed from the cast-off outer layer of the cuticle of the nematode, but it is covered with lymph-cells, some of which usually remain attached to it even after the disintegration of the brown body as a whole. It is seldom extended straight. The commonest shapes in which it is bent are the figures 3 and 8, and it is often coiled in a ring or a spiral (Pl. 37, fig. 5).

I have seen the cœlomic form in the act of emerging from the cyst on numerous occasions. One end of the cyst—usually the anterior end—is ruptured or pushed off as a cap, and the nematode works its way out by a prolonged series of writhings and contortions. Among the constituents of the disintegrating brown bodies, nematodes, covered with amœbocytes but with the cysts not yet formed underneath these, are frequently to be seen.

In addition to the nematodes congregated in the brown bodies at the tail, independent encysted individuals surrounded by cœlomic corpuscles are found in smaller numbers in all parts of the cœlom. Unencysted individuals also occur. The cœlomic form is sometimes found imbedded in the muscular layer of the body-wall or encysted on the septa (one of the positions given by Schneider, who does not mention the brown bodies in the tail and may not have seen them). Lankester (**4**, Pl. vii, fig. 12) gives a drawing of a nematode in the former position.

I have examined other species of earthworms besides Lumb. terrestris, the common earthworm, and have found the brown bodies in Lumb. rubellus Hoffmeister, Allolobophora longa Ude, All. turgida Eisen, and Octolasium cyaneum Savigny, and I do not doubt that they occur in other species as well. All except those in All. turgida contained encysted larval nematodes.

Though varying considerably in size and stoutness of build, the cœlomic form is always found in a larval, never in an adult, state in the freshly killed worm.

(2) The Active Form living free in the Nephridia.

The nephridial form inhabits the cavity of the "bladder," the dilated muscular termination of the nephridial tube next to the nephridiopore. It occurs very constantly in worms of this particular species. Several are to be found in almost every nephridium. The number present varies from two or three to over a dozen. The nematodes are found in worms of all sizes. The largest and most healthy-looking appear to be infested quite as much as the weakly specimens. I have seen the nematodes in worms which are only 1·5 in. long and are so young as scarcely to be recognisable as Lumb. terrestris. The only part of the nephridium in which the nematodes occur is the bladder. On one occasion, certainly, I saw one in the "wide tube," but it had evidently strayed from the bladder and it soon went back.

The active form is sometimes met with in the seminal vesicles (5, p. 11).

I have examined other species of earthworms besides Lumb. terrestris, and have found the nephridial form present in Lumb. rubellus Hoffmeister, Eisenia fœtida Savigny, Dendrobæna subrubicunda Eisen, and Octolasium cyaneum Savigny. They are in some cases plentiful, as they are in Lumb. terrestris. But they are more often present in only very small numbers, and most frequently absent altogether. The nephridial form in Oct. cyaneum belongs to the same species as that in Lumb. terrestris. I have not identified those found in the other species of earthworms, but I believe them also to be the same.

The nephridial form is in an active condition, though it may exhibit very varying degrees of activity. It often remains coiled and motionless or makes only sluggish movements. At other times it writhes unceasingly. Like the cœlomic form it is always found in a larval, never in an adult, state in the freshly killed worm. Like the cœlomic form, too, it varies considerably in size and stoutness of build, but the majority of individuals are smaller and slenderer. Its average length is ·5 mm. (Pl. 37, fig. 4). The two forms appear identical in structure and proportions. But, in the undeveloped condition in which they exist in the worm, they do not exhibit features sufficiently distinctive to make identification possible, the different species of Rhabditis being very much alike in the larval stage. The sexually mature males provide the most important means of discriminating the different species, the disposition of the papillæ or rays of the bursa (Pl. 37, fig. 9) being the most useful diagnostic character.

In order, then, to determine the identity of the nephridial form it was necessary to rear it from the larval to the adult condition, and with this object, as well as with that of investigating the sexual phenomena, cultural methods were employed.

Cultural Methods.

The researches of Maupas (**10**, **14**), extended by those of Potts (**15**), on the free-living nematodes have shown the possibility of rearing different species of Rhabditis, Diplogaster, Cephalobus, etc., in artificial media. The method employed by them consists in the use of watch-glasses, preferably of the "solid" kind, in which the nematodes are kept in drops of water to which is added a small quantity of the nutritive medium. To prevent evaporation of the medium the watch-glasses may be closed by glass covers fastened down with vaseline, or a number may be placed together, without covers, in a humid chamber. The latter alternative is employed in cases where the decomposition of the medium is so intense that the nematodes would succumb to the effect of the gases liberated in putrefaction, were they confined in the small space afforded by the cavity of a single watch-glass. By this method of culture nematodes can be maintained in conditions favourable to growth and reproduction, and, given a suitable medium, generation after generation can be reared merely by transferring a mature female of one generation into a fresh watch-glass, in which it may become the parent of a further generation.

The temperature at which the cultures were carried on was the ordinary temperature of the laboratory.

For the liquid in the watch-glasses into which the nematodes were put ordinary tap-water or salt solution was used. It was first filtered to remove any nematodes which might be present in it. The culture medium was then added, and replenished afterwards from time to time as required.

If, as often happened, the medium in the watch-glass became too cloudy to allow direct examination of the nematodes under the microscope, they had to be removed singly on the point of a needle or a few at a time by means of a fine pipette, and transferred to a drop of water on a slide. Nematodes lying in water or in the nutritive medium in a watch-glass can be examined under the microscope with a low, but not with a high power. For examination with the high power they must be transferred to a slide.

Various media have been employed. They are here given. roughly in the order in which they were tried. Most of them were abandoned as useless, and none have proved altogether satisfactory. The nematodes inhabiting the worm were found exceedingly difficult to rear. From some cause not yet understood, whole cultures have died off quite suddenly and unexpectedly, breaking the continuity of experiments and rendering it in many cases impossible to obtain conclusive results. The explanation for this may perhaps lie in the peculiar conditions in which these nematodes live. But, in addition to the sudden and unaccountable deaths of whole broods of nematodes in cultures which began promisingly, great difficulty was frequently experienced in the starting of cultures. Again and again every one of the larvæ removed from the nephridium died as soon as introduced into the food-medium. The difficulty of maintaining the cultures in a healthy state was on the whole not so great as that of making a successful start.

A nearly saturated solution of peptone (Witté's) was tried but this strength proved useless, as the nematodes died soon after being placed in it. Weaker strengths were then tried, with the same results, until a ·15 per cent. solution was reached. This, though exceedingly dilute, proved satisfactory for one series of cultures. But after that peptone was almost uniformly unsuccessful and was abandoned.

Hay infusion (1 per cent.), like peptone, was successful for one series of cultures, but in other cases was unsatisfactory. Hay infusion, first sterilised and then inoculated with soil bacteria, was no improvement. A solution of urea was tried but was found to be useless. Meat extract and decaying meat were also unsatisfactory. Since these artificial media were unsuccessful, the natural food of the nematodes was used instead.

Earthworms decaying in damp soil were tried, as being the natural medium in which Rh. pellio develops. The mode of procedure is as follows. A freshly killed Lumb. terrestris containing nematodes in the nephridia and cœlom is opened, and its gut is removed in order to obviate any chances of contamination with soil nematodes which may happen to be passing through in the soil which has been swallowed. The nephridia and the brown bodies in the cœlom are left remaining. The worm is placed with soil in a watch-glass and then moistened with water. The soil is first heated to kill off any soil nematodes in it. But the temperature required to make certain of having done so probably renders it sterile and therefore valueless. At any rate, I find that it can be dispensed with. Within a week the decaying worm is seen to be covered with a whitish mass of actively writhing nematodes. Examination shows these to be adult males and females, the latter being the larger. Several generations are produced before the nutriment provided by the rotting carcase of the worm is exhausted. On comparing these adults obtained from the putrefying worm with those reared in peptone or hay infusion the difference in size is seen to be very striking (Pl. 37, figs. 2 and 3). The putrefaction form is considerably the larger and stouter. Further, its reproductive organs are larger than those of the peptone form in proportion to the rest of the body, and the eggs in the uteri of the females are much more numerous, although, strangely enough, they are individually smaller. Decaying earthworm is of higher nutritive value than peptone, but it is in several respects less useful as a food-medium. Unless specially treated beforehand it cannot be employed for nematodes which are required to be reared in isolation, for the body-wall already teems with nematodes from the nephridia and cœlom. In order, therefore, to remove these I proceed as follows.

A Lumb. terrestris is killed, and from along the whole length of the worm is cut a narrow strip of the dorsal part of the body-wall, a region to which the nephridia, infested as they are with nematodes, do not usually extend. To insure the entire removal of the nematodes imbedded in, or encysted on, the body-wall or present in any nephridia that may be left attached to the strip, the greatest care is exercised. The strip is laid on a slide and kept moist with water for about two days. During this period it is examined under the microscope from time to time. The nematodes which are present on the strip of body-wall congregate in the water round it, the encysted ones emerging from their cysts as it begins to putrefy. Unless the strip is heavily infested they can almost all be removed on the point of a fine needle. The relative thinness of the body-wall allows a fairly minute examination of it as a semi-transparent object, so that nematodes still left buried in the muscular tissue can be dug out with a needle, unless too deeply imbedded. When they have all been removed the strip is ready for use. A piece is cut from it and placed in a watch-glass with a small quantity of water and the nematode which it is required to cultivate. A control, consisting of another piece of the same strip moistened with water, is kept in order to make certain of the entire absence of nematodes from the medium employed. Decay is rapid, and the nematodes in the culture, like those reared on the body-wall decaying in its entirety, attain large dimensions. While sharing with the latter medium this advantage over peptone, the new form of medium has also certain drawbacks in common with it. The body-wall, as it becomes more and more opaque in the course of decay, makes a dark background on which it is difficult to distinguish the nematodes in the culture. Occasionally, also, the whole contents of the culture develop an evil odour or in addition become clouded over with a dense scum, not only rendering examination impossible, but also causing the death of all the nematodes. But the chief drawback, and the one which rendered it necessary to discontinue the use of the strip method, was the presence of nematodes which were found infesting the medium in certain cases even after the exercise of the utmost care in attempting to remove them all.

A third medium, designed to avoid the disadvantages of its predecessors, has been devised and is prepared as follows :

Several Lumb. terrestris are killed and then cleaned by the removal of the gut. They are put with some water into a test-tube, which is then plugged with cotton-wool, heated in a steamer and kept at boiling-point for about two hours. The opaque broth is decanted and filtered. The almost clear resultant liquor, in which putrefaction very soon commences, is inoculated with the bacteria, which in the natural state are associated with the decay of the worm in the soil. For this purpose a freshly killed Lumb. terrestris is allowed to decay in a small quantity of water to which a little earth is added, and a spot of this fluid, carefully examined under the microscope to get rid of any nematodes in it, is added to the clear liquor before the bacteria from the air have had time to multiply in it. The liquor soon becomes cloudy with the growth of the soil bacteria and is then ready for use. This worm extract has been used for all the most recent cultures. It forms on the whole an excellent food-medium. Certainly it is the best that has yet been devised. Like the body-wall medium, it is more nutritious than peptone, and produces adults of large size. Over decaying body-wall it possesses the twofold advantage (for the sake of which it was devised) of not being liable to contamination, and, of affording a fairly clear view under the microscope of the nematodes under cultivation. It is open, however, to the same objection as the body-wall medium. Occasionally an evil odour develops, accompanied as a rule by the appearance of an opaque scum, and all the nematodes in the culture die off.

The dimensions to which the adult nematodes attain vary according to the nutritive quality of the media on which they are reared, their size being directly dependent on nutrition. Thus those fed on worm extract or putrefying worm (the natural food) considerably outgrow those fed on peptone, and the latter cannot be regarded as typical of the species in regard to size.

The length of life of these nematodes in a natural state will be considered later in connection with their life-history. In artificial cultures, the period which the larva occupies in reaching maturity after its removal in the nephridial stage from the worm corresponds of course to that part of the nematode's life which in a state of nature elapses between the death of its host in the soil and its appearance as an adult. During this period growth is exceedingly rapid. In worm extract maturity is usually reached in four days. But occasionally mature females have been obtained in three days and males in two. Decaying body-wall takes about four days, peptone about six. Another period, easily ascertained in cultures, is that which elapses between the hatching of embryos from the eggs of successive generations of nematodes. In worm extract this is about eight days, in peptone about ten. While the period of growth prior to reproduction is fairly uniform, the length of life of the adults kept in cultures after reproduction is very variable. Some females succumb to the effort of reproduction, others survive the process by only a few days, and others live on afterwards for two or three weeks.

The use of an artificial culture medium shows that the distinction between oviparity and viviparity as specific characters does not exist in these nematodes of the earth-worm. They are oviparous or viviparous according to the food supply. Those fed on peptone are always oviparous. Those fed on putrefying worm are usually oviparous at first, but, since they produce a far larger stock of eggs than the peptone form, they become viviparous later, when the eggs are being discharged into the uterus faster than they can be laid. The embryos are ready to emerge before the eggs have been laid, and therefore they hatch out in the uterus, and, when discharged from the parent's body, are no longer enclosed in the egg-shell. The nematodes of the earthworm are thus oviparous or viviparous according to the nutritive quality of the food supply. But both the peptone form and the putrefaction form behave, so to speak, viviparously if they die before all their eggs are laid, for the fertilised eggs that remain in the uterus, being quite independent of the parent for their nourishment, continue their development, and the embryos hatch out within the uterus of the parent, in whose decaying body they thrive and grow until nothing remains but a thin investment of cuticle.

Identification.

The encysted larvæ from the cœlom are reared to the adult stage (at which they can be identified) in the following manner. A number of the brown bodies from the tail of a freshly killed Lumb. terrestris are placed in a watch-glass with a little water. Disintegration and decay set in very soon, providing a natural food medium for the nematodes which emerge from the cysts. They grow rapidly, and within a week have developed into sexually mature adults of Rhabditis pellio, exactly similar to those obtained from the worm decaying in its entirety (Pl. 37, figs. 1 and 2).

This, however, is merely a confirmation of the results obtained by the early investigators. The question which the literature of the subject does not appear to have hitherto answered is, whether the active larvæ in the nephridia are the same species as these or not? I have not discovered that any previous worker has ever removed the larvæ from the nephridia and reared them to maturity in order to establish their identity, and therefore I have done this myself. Larvæ from the nephridia of a Lumb. terrestris were put in a watch-glass with small strips of decaying body-wall and a little water. Growth was rapid. When sexually mature they were examined and were found to be Rhabditis pellio.

The nephridial and cœlomic larvæ, therefore, are the active and encysted forms respectively of one and the same species. This has been confirmed by all subsequent cultures. Further, I have found no other species but this inhabiting the living worm.

Confusion of Two Species under One Name.

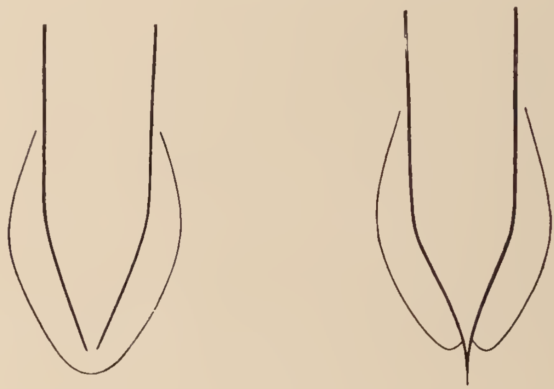
A perusal of the literature on Rhabditis pellio leads one to suppose that all the nematodes which have been encountered in connection with the earthworm and described under this name are genuine inhabitants of the healthy worm and belong to a single species. But whether this is really so, and whether the name does not require to be more clearly defined, are questions which I propose to discuss.

I have mentioned earlier in this paper in the survey of the literature that Bütschli (**6**) noticed certain differences between the adult individuals of Rh. pellio which he examined and those which had been described by Anton Schneider (**1**). There were various minor discrepancies. But the most striking point of dissimilarity was that the tail of Schneider's males did not project beyond the edge of the bursa, while that of Bütschli's males was prolonged to a fine point a short distance beyond the margin, which was slightly notched at the place of emergence.

Bütschli regarded these differences as unimportant, and supposed that the two different forms of bursa merged into one another by imperceptible gradations. Subsequent references to Rh. pellio in the literature of the Nematoda are for the most part very meagre. With the exception of Maupas, the writers do not comment on the dissimilarity of the two forms. Maupas (**10**), however, twenty-six years after the publication of Bütschli's work, wrote as follows: "Sous ce nom de Rhabditis pellio on confond deux espèces distinctes: 1° le type décrit par Schneider ('Monographie der Nematoden,' p. 154); 2° celui décrit par Bütschli ('Beiträge zur Kenntniss der freilebenden Nematoden,' p. 112); la première espèce est une forme pélodérienne, la seconde une forme leptodérienne. . . ." (Text-fig. 1.)

In support of this statement that the two forms are

Text-fig. 1.



"Peloderian" tail. "Leptoderian" tail.

distinct species, there is, I think, convincing evidence Firstly, Maupas says: " . . malgré l'opinion contraire du Bütschli, je me suis convaincu que cette absence ou cette existence d'un prolongement caudal mâle constitue bien un excellent caractère distinctif. J'ai eu occasion d'observer des milliers d'individus des deux types, obtenus dans des cultures isolées, et jamais je n'ai vu ce caractère faire defaut." Secondly, neither Schneider nor Bütschli saw the form described by the other, and Bütschli does not say he ever saw the intermediate forms which he supposed existed. Thirdly, in my own case, I have never found any but Bütschli's "leptoderian" form. The "peloderian" form of

Schneider and the "leptoderian" form of Bütschli are clearly distinct and separate species.

Now Bütschli's "leptoderian" adults are the form developed from the larvæ living in the worm, as my own results have abundantly shown. Schneider's "pelodorian" adults, on the other hand, were obtained from worms decaying in soil, and were not actually proved to have been developed from the larvæ inhabiting the worm. Further, while those of my cultures of decaying worms, in which all chance of contamination from soil larvæ has been excluded, have never yielded any but the leptoderian species, those, on the other hand, in which earthworms are allowed to decay in ordinary soil have, as von Erlanger also (9) has shown, yielded others besides the "leptoderian" species. Indeed, on one occasion, in examining some earth in which several Lumb. terrestris had died and decayed, I found a number of larval nematodes, from which, when reared in worm extract, I obtained a male with a "pelodorian" bursa and the bursal papillæ disposed, as far as I can judge from a rough drawing made at the time, similarly to those of Schneider's form. These considerations afford strong evidence that Schneider's "pelodorian" form was a soil-inhabiting species, attracted while larval to the decaying worm, on which it developed and matured. This would be quite in keeping with the behaviour of the free-living species of nematodes inhabiting the soil, studied by Maupas and Potts.

On this hypothesis the reason why Schneider made the mistake of supposing his "pelodorian" form to have developed from the nematodes inhabiting the worm as larvæ is not far to seek. Lieberkühn had seen the larval nematodes in the worm develop on the decay of the latter into sexually mature adults (which, however, he did not describe), and Schneider would naturally suppose, since he did not actually make the experiment and prove the contrary, that the adults which he himself found on the decaying worm had likewise developed from these larvæ. In the second place, the reason why Bütschli failed to recognise that the form which he described was a distinct species from that of Schneider was evidently because Schneider's form, like his own, had occurred on decaying worms, and he did not realise that the presence of soil allows chances of contamination with soil-inhabiting forms.

It may be asked why neither writer recorded the form described by the other. The explanation may be that Bütschli probably did not find his infected earthworms decaying in soil like Schneider's, but killed them himself and allowed them to decay in water, in which case the "peloderian" form would not be present; and that Schneider made his description from only a few individuals, amongst which none of the "leptoderian" species happened to be included.

With the notable exception of Maupas, later writers, as I said before, do not comment on the difference of the two forms. The probable reason was that they saw Bütschli's "leptoderian" form only, because soil was excluded from contact with the putrefying worms which they used, or they may have seen both forms but did not suspect them to be distinct species because they did not recognise that the soil is a source of contamination.[1]

In view, then, of the confusion, under the same name, of two undoubtedly distinct species found in the same situation in the adult state but derived probably from different larval habitats, I propose to distinguish between them by narrowing the application of the name. I propose to restrict the name Rhabditis pellio Schneider to the "peloderian" form described by Schneider, and provisionally to designate the

[1] Since writing the above I have been able to consult Örley's 'Die Rhabditiden und ihre medicinische Bedeutung,' Berlin, 1886 (14). The tail of the male Rh. pellio which he describes (p. 33) is of the "peloderian" type and closely resembles that of Schneider's form. But whether he made his description from adults bred, without risk of contamination with soil nematodes, from larvæ actually inhabiting the living worm, and not from adults obtained from dead worms allowed to decay on soil (his method of obtaining large numbers of nematodes) appears to be doubtful.

leptoderian form, described by Bütschli, but wrongly regarded by him as belonging to the same species, by the name Rhabditis pellio Bütschli, non Schneider. No doubt the correct course would be to call the "leptoderian" species by a new name, but until Schneider's "pelodorian" species has been re-examined, I hesitate to take this logical step.

In reading this paper no confusion should, I think, be caused by this splitting of what has hitherto been regarded as one species. This paper deals exclusively with Bütschli's "leptoderian" form, which I call Rhabditis pellio Bütschli, non Schneider, the species whose larvæ, both free and encysted, infest the living worm, and it is this form which is intended when the abbreviation Rh. pellio B. is used.

Anatomy.

It will be well at this point to say something of the anatomy of Rhabditis pellio B. since the descriptions given by Schneider, Bütschli and von Linstow are not quite complete.

The accompanying table of measurements is intended to show the inferiority in size of the adult reared on peptone to the typical form fed on decaying worm. The arrangement is based on the admirable system used by Maupas (**15**). The fractions express the relation between the length of the particular region and the total length of the body. The length given for the buccal cavity, however, is relative to the total length of the œsophagus, of which it is regarded as part. The measurements of the adults—subject as they are to great variation—have been taken as far as possible from the largest individuals obtained.

The cuticle is transparent, and marked by exceedingly delicate transverse striations difficult to distinguish except at the anterior and posterior ends.

The mouth (Pl. 37, fig. 6) is bordered by three lips, one dorsal and two ventral, each of which is divided by a shallow groove into two lobes. Each lobe bears a pair of fine short papillæ. The buccal cavity in side view appears to be cylin-

Table I.—Measurements.

	Adult female.		Adult male.	
	Typical form.	Peptone form.	Typical form.	Peptone form.
Length . .	2000 μ	1238 μ	1781	900 μ
Œsophagus . .	$226 = \frac{1}{9}$	$248 = \frac{1}{5}$	$209 = \frac{1}{9}$	
Tail . . .	$150 = \frac{1}{13}$	$143 = \frac{1}{9}$	$72 = \frac{1}{25}$	$64 = \frac{1}{14}$
Vulva . . .	1019	638		
Diameter . .	$143 = \frac{1}{14}$	$86 = \frac{1}{14}$	$104 = \frac{1}{17}$	$50 = \frac{1}{18}$
Buccal cavity .	$24 = \frac{1}{9}$		$23 = \frac{1}{9}$	
Spicules . .			67	49
	Larva in Nephridium.		Egg.	
			Typical form.	Peptone form.
Length . .	510 μ		56 μ	62 μ
Diameter . .	$28 = \frac{1}{18}$		$37 = \frac{1}{1 \cdot 5}$	$47 = \frac{1}{1 \cdot 3}$

drical. It is of equal width throughout, except at its posterior end, just in front of the point where it is continued into the œsophagus. There it narrows and then widens again, ending in a thickened edge. The œsophagus has two bulb-like swellings, an anterior elongated one and a posterior rounded one, the latter bearing a dental armature. A part of the anterior end of the œsophagus is reflexed forwards round the posterior three fourths of the buccal cavity. The intestine (Pl. 37, fig. 2) is a tube formed between two rows of large alternating cells containing large nuclei. Its dark appearance by transmitted light is due to the granules deposited in its walls. The lumen of the intestine, and also of the whole of the alimentary canal, is lined with chitin. The rectum

(Pl. 37, fig. 8) is exceedingly short and has a narrow cavity. It can be distended by means of muscular fibres passing to the body-wall. Its anterior end is surrounded by three unicellular glands, two lateral and one median dorsal. The anus is ventral, and appears to be a transverse slit situated at the base of a papilla.

The nerve collar (Pl. 37, fig. 6) surrounds the œsophagus between the anterior and the posterior bulb. It is inclined obliquely in an antero-ventral direction.

The excretory canals (Pl. 37, fig. 6), which are difficult to see, pursue a sinuous course in the body-wall along either side. From each canal a single downward branch descends towards the excretory pore. Bütschli (**6**, pl. x, fig. 59e) represents these as uniting before reaching it. The excretory pore is situated in a median ventral position a short distance behind the posterior œsophageal bulb.

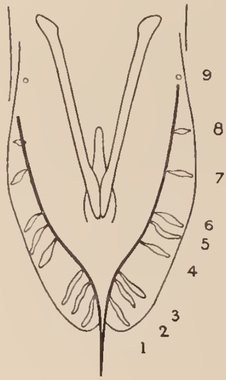
The female is long and narrow and tapers gently towards either end.

The tail of the female (Pl. 37, fig. 7) is prolonged to a fine point. It bears a pair of short papillæ, one on each side, a little more than a third of the distance backwards from the anus. They do not project from the surface of the body, but are continued inwards, each as a delicate fibre running forwards towards the anus. They are not always exactly opposite one another.

The vulva, the reproductive aperture of the female, is placed slightly more than half-way along the body in a median ventral position, and has two projecting lips bordering a transverse slit. The vagina is short. In females of the typical form reared on putrefying worm the paired uteri are long and distended, and contain probably between one and two hundred eggs (Pl. 37, fig. 2). In the form fed on peptone they are smaller and contain five to twelve eggs (Pl. 37, fig. 3). The ovaries are long and reflexed dorsally, nearly reaching back to the vulva. The so-called "seminal receptacles," which are really the oviducts, are filled with spermatozoa, the female of this species reproducing as a hermaphrodite. They are short, narrow tubes connecting the ovaries with the uteri and bent in the form of an S or a U. The eggs are oblong-oval. Those of the typical putrefaction form are 56 μ in length and 37 μ in diameter, while those of the form reared on peptone, though so much fewer in number, are 62 μ in length and 47 μ in diameter.

The bursa (Pl. 37, fig. 9), the copulatory organ of the male, is broadest in the middle. In all the males that I have seen the tail is prolonged a short distance beyond the end of

Text-fig. 2.



Tail of a male Rhabditis pellio B. as seen in ventral view, showing the disposition of the bursal papillæ.

the bursa, whose edge is emarginated at the place of emergence. There are nine papillæ, disposed in groups of three each (Text-fig. 2). The posterior three are close together. Counting from the posterior end the second papilla is usually nearer to the third than to the first. The median three are also close together, and separated from the posterior group by an interval. In the median group the fifth papilla is usually nearer to the sixth than to the fourth. The interval between the anterior and median groups is equal to that between the median and posterior groups. In the anterior group there is a somewhat wide interval between the seventh and eighth, and a very wide interval between the eighth and ninth papillæ.

There is a great deal of variation in the disposition of the bursal papillæ in different individuals, even of the same brood. Single papillæ may be missing, extra ones may be present, or the usual intervals between papillæ may be altered. These variations may occur on one side of the bursa only, or on both. The fifth papilla may be nearer the fourth than the sixth, or the intervals between the fourth, fifth and sixth, or between the seventh, eighth and ninth may all be equal.

The papillæ do not reach the edge of the bursa but bend round, their ends pointing perpendicularly away from its ventral surface. The copulatory spicules (Pl. 37, fig. 9) are strong, slightly curved and thickened at their anterior ends. They are structurally independent of each other but work in unison. The accessory piece (Pl. 37, fig. 10) is a little more than half the length of the spicules. The testis (Pl. 37, fig. 1) is full of spermatozoa in different stages of development. The vas deferens is a tube formed of large cells with small nuclei. The lumen is narrow. The spermatozoa are oval and granular with a refractive nucleus of irregular outline.

Questions of Sex.

(1) Analysis and Character of Reproduction.

The work of Maupas (**14**), supplemented by that of Potts (**15**), has shown that in Rhabditis and several closely related genera the mode of reproduction is by no means uniform. At first it was thought that all species were bisexual. It is now known that side by side with the bisexual species, in which males and females are produced in equal numbers, hermaphrodite and parthenogenetic species also exist.

In the hermaphrodite species, which appear to be even more numerous than the bisexual species, the females reproduce as self-fertilising protandrous hermaphrodites and constitute the bulk of the individuals, as a rule vastly outnumbering the males, which in some species are almost entirely wanting. The males, though apparently as perfectly developed as those of the bisexual species, yet take no part in the reproductive process or only "re-fertilise" the females on rare occasions when the latter have exhausted the stock of spermatozoa produced in their genital organs. It would appear quite permissible to apply the term "female" to the reproductive individuals of the hermaphrodite species. They closely resemble the true females of the bisexual species of the same genera, the only essential anatomical difference being the presence of spermatozoa developed in the oviducts, which are hence called the "seminal receptacles." Indeed, there is evidence that the conversion of females into hermaphrodites is a process which is going on at the present day.

In the parthenogenetic species the females do not develop spermatozoa, and males are entirely absent.

So far as the question of its reproduction has received any attention at all, Rhabditis pellio appears to have been hitherto regarded as a purely bisexual form. But whether this is the case and whether the species is not somewhat variable in its sexual character are questions on which I hope the following investigation will throw some light. The males have never at any time been seen to take part in the reproductive process or even to exhibit any sexual instincts whatever, nor are they numerically equal to the females, as in the bisexual species.

When dead earthworms decay, the male and female nematodes which develop from the larvæ in the nephridia and cœlom propagate readily. But when larvæ were removed from a freshly-killed worm and placed in an artificial medium, the proportion of productive adults was in most cases considerably smaller, the medium being for some reason less favourable to reproduction than the natural food. This must be borne in mind in considering the following cultural experiments :

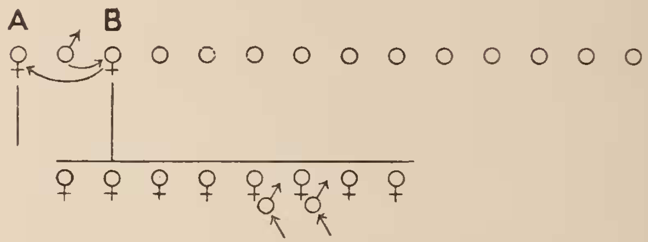
Culture A.—Two sexually mature females, which may or may not have been already fertilised by males, were removed from some decaying brown bodies of a Lumb. terrestris and isolated in worm-extract in watch-glasses. Both produced young. Both broods grew up into females (= F_1 generation). No males were produced. One young female was isolated in worm-extract while still larval. It matured and reproduced, but both it and its offspring died immediately afterwards. In this culture the isolated female of the F_1 generation unquestionably reproduced without being fertilised by a male, for not only were there no males in the brood from which it was taken, but it was still larval when isolated.

Culture B.—Two larvæ were removed from a nephridium and isolated in watch-glasses with peptone. Both developed into females, which matured and propagated. A series of about eight generations was produced. (The exact number was not recorded.) Amongst all the offspring of the various generations no males were found. The females of this culture, therefore, like the isolated female of the first filial generation of Culture A, propagated without any males being present to fertilise them. Spermatozoa were distinguished in the "seminal receptacles." Parthenogenesis being thus ruled out, reproduction must have been hermaphrodite.

Culture C.—Several nephridia were removed, together with the larval nematodes which they contained, from a freshly killed L. terrestris into a watch-glass with a little water. As the nephridia decayed the larvæ rapidly matured and reproduced. An F_1 and an F_2 generation were produced, and both were cultivated in worm-extract. In this culture, males, besides being present in the parental generation, were also produced in each of the filial generations. There were no cases of undoubted hermaphrodite reproduction, that is, in no instance did females which had been isolated while larval propagate offspring. Only those females which were kept with males reproduced. This is strong evidence for bisexuality, but is not decisive, since the females placed with the males may not have been fertilised by them, but may have produced their own sperm as hermaphrodites, and the reason why the isolated females refused to reproduce may have been, not the absence of males, but the poorness of the food-medium. This objection receives support from the fact that, although only those females which were placed with males reproduced, yet by no means all, but only a small percentage of them, did so.

Culture D.—Several nephridia, with the nematodes which they contained, were removed wholesale from a freshly killed Lumb. terrestris. Fourteen of the larvæ were picked out and isolated in watch-glasses with worm extract. All but three died while larval, before their sex was indicated. (I represent such individuals in the culture-table by a plain circle. Of the three survivors two developed into females and one into a male. The one female, which I shall call A, began to lay disintegrating eggs. (Disintegration is a sign that the egg has

TABLE 2.—CULTURE D.



not been fertilised.) The male was put with the other female, B, which had not yet laid any eggs at all. Next day B began to lay fertilised eggs, from which larvæ (F_1 generation) hatched out. The male was then removed from B and placed with A, which had by this time ceased to lay even sterile eggs. Two days later A also began to lay fertilised eggs, from which larvæ (F_1 generation) hatched out.

Eight larvæ were isolated from among the offspring of B. All developed into females. So did all the unisolated larvæ of the same brood and also A's brood. None of the eight isolated females reproduced, even though extraneous males, taken from a culture of the nephridial form reared on decaying nephridia, were placed with two of them. Nor did the unisolated females of A's or B's broods reproduce.

The behaviour of the parental generation in this culture affords the strongest evidence for bisexuality that I have yet been able to obtain. The evidence is not absolutely conclusive, since the males have not actually been seen to fertilise the females either in this case or in any other, but it is very strong. The refusal of the isolated females of the F_1 generation to reproduce is additional evidence. But, on the other hand, the two females of this generation with which the extraneous males were placed still remained unproductive.

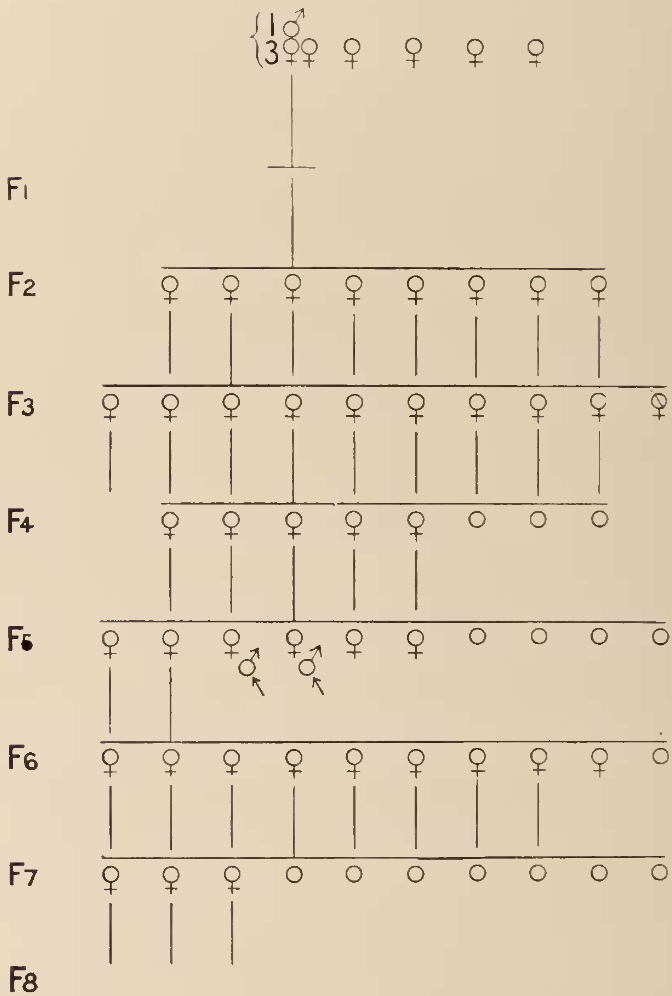
It should be noted that, if the male did in this culture actually take part in reproduction, it would appear that a male is capable of fertilising more than one female of the same generation.

Culture E.—Eight larvæ were taken from the nephridia of a freshly killed Lumb. terrestris and isolated in worm extract in watch-glasses. Seven developed into females and one into a male. Four of the seven females were kept isolated, and the male was placed with the remaining three females. On examining the cultures after an unavoidable absence, I found that, while the four isolated females had laid only unfertilised eggs (which rapidly disintegrated), the three females placed with the male had produced an F_1 generation, which in its turn had given rise to an F_2 generation. The parents and the first and second filial generations were all together in the same watch-glass, but could be distinguished by their different sizes. The F_2 generation was larval and quite small. In the F_1 generation I could see no males. The only male to be seen was the parental individual. Those females of the F_1 brood, therefore, which reproduced probably did so as hermaphrodites, unless fertilised by the parental male, which I do do not think probable.

Eight of the small larvæ belonging to the F_2 generation were isolated. All developed into females, nor were any males seen among the unisolated remainder. Each of these eight females produced an F_3 generation. Their mode of reproduction was unquestionably hermaphrodite, for they were isolated while in the larval condition.

Ten larvæ of the F_3 generation were isolated from one of the most prosperous of these eight broods. All grew up into

TABLE 3.—CULTURE E.



females, nor did I see any males among the un-isolated remainder. All the females reproduced except one, which died before it could reproduce. I represent such an individual by the female sign with its circle crossed by a line, to distinguish it from those which live long enough to reproduce but are for some other reason unproductive.

The culture was continued on the same lines until it died out in the F_8 generation.

Regarding the isolated unproductive females as forming a control, the parental generation in this culture appear to have reproduced bisexually. The F_1 generation reproduced probably as hermaphrodites. The F_2 and all subsequent generations reproduced unquestionably as hermaphrodites. No males were produced in any of the broods. Extraneous males, taken from a culture of the nephridial form reared on decaying nephridia, were placed with two unproductive females of the F_5 generation, but did not induce reproduction.

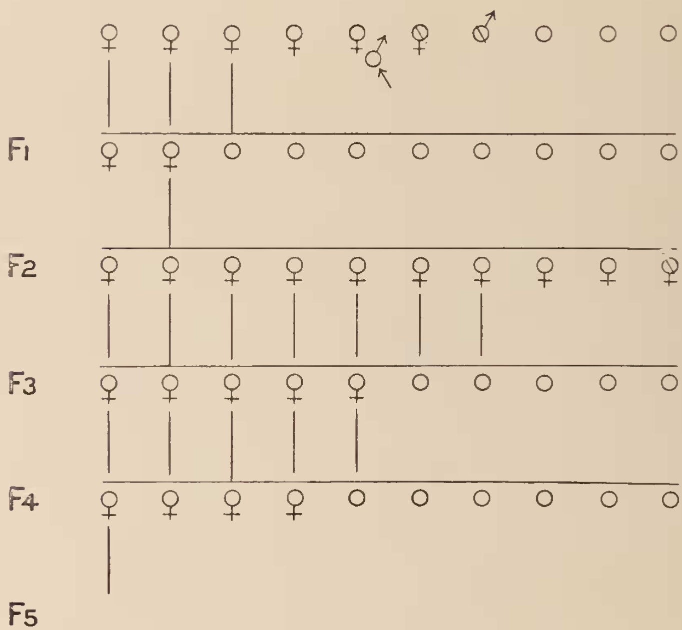
Culture F.—Ten larvæ were taken from the nephridia of a freshly killed Lumb. terrestris and isolated in worm extract in watch-glasses. Three died while larval. Of the remaining seven six developed into females and one into a male. The male and one female were killed accidentally. Of the other five females three reproduced but two did not, although an extraneous male taken from a culture of the nephridial form in decaying nephridia was placed with one of the latter.

Ten larvæ of the F_1 generation were isolated from one of the three broods. Eight died while larval. The remaining two grew up into females, nor did I see any males among the unisolated remainder. One of the two females reproduced, the other did not. The culture was continued on the same lines until it died out in the F_5 generation.

In this culture reproduction was hermaphrodite in all cases. Nor were males seen in any of the broods. Further, this is the only case in which reproduction has been unquestionably hermaphrodite in the parental generation.

Conclusions.—From the numerous cases of reproduction by the females in the absence of males and the finding of spermatozoa in the genital organs of such individuals, it is clear that the females are often—if not always—hermaphrodite. On the other hand, in spite of the fact that males have never been observed to exhibit any sexual instincts whatever, it seems probable, from the cases, especially in the parental generation, in which the females have propagated only when males have been present, that reproduction is sometimes bisexual. This

Table 4.—Culture F.



would mean that a number of true females exist side by side with the hermaphrodites as in *Rh. marionis*, a species described by Maupas (**14**, p. 506).

Rh. marionis affords also a remarkable instance of incipient hermaphroditism. Among the hermaphrodites not only are true females occasionally found, but also individuals which are partly hermaphrodite and partly female, the one half of the paired genital gland producing both eggs and sperm, the other half giving rise to eggs only. The males also occasionally "re-fertilise" hermaphrodites whose stock of spermatozoa has become exhausted. In the present species, however, I did not see any of these partially hermaphrodite individuals or discover any cases of re-fertilisation.

It may be asked why bisexuality does not, like hermaphroditism, admit of experimental proof, without actual observation of the males in copulation with the females. The reason is that the results of an experiment may be misleading. A female placed with a male may reproduce, whilst a female, isolated when larval as a control, may not. This result might at first be regarded as an almost undoubted case of reproduction. But the real facts may be that the female placed with the male reproduced as a hermaphrodite, developing spermatozoa for itself and not receiving them from the male, in spite of appearances to the contrary, while the isolated female refused to reproduce, not because of the absence of males, but from some other cause. It should be mentioned that in this species, on account of the position of the gut, it is often very difficult, especially in young or unproductive females, to distinguish whether spermatozoa are present in the reproductive organs or not.

(2) The Sex Ratio.

In regard to the sex ratio the results of the foregoing cultures and other observations not recorded here work out as follows. The nematodes removed from the freshly killed worm and reared in cultures, or obtained from the dead worm decaying under natural conditions, develop into males and females. Subsequent generations, however, bred under cultural conditions consist in almost all cases of females only. This difference between the ratio of males to females in the parental and in the filial generations under cultural conditions is most striking. In the parental generation the ratio of males to females was about 1 to 5, which for a hermaphrodite species is very high. After this the ratio was, with few exceptions, so low that in the restricted number of generations through which the cultures lasted no males were seen at all. In a few instances, however, males did occur in the filial generations, and in such cases the ratio was sometimes high. The first filial generation of culture C, which consisted of 36 males and 31 females, furnishes a very striking instance of this.

Potts (**15**), in reference to the free-living hermaphrodite species which he studied, states that the production of males is cyclical, but that there is no rule that they should appear at stated intervals or restricting their production to a period or periods of maturity. My own cultures did not last long enough to enable me to throw light on the question. But, as has been already stated, although males are as a rule entirely wanting in the filial generations, they always develop in a high ratio in the parental generation consisting of larvæ taken from the freshly killed worm; and it is not unreasonable to suppose that, had the cultural series lasted over a longer period, males would sooner or later have reappeared.

Potts states that it is not probable that the sex proportions are governed by nutrition, and my own results bear out this opinion.

Maupas (**14**), in inducing males of Rh. elegans (p. 477) to re-fertilise hermaphrodites whose stock of sperm was exhausted, obtained an enormous increase in the proportion of males to females in the offspring, so that the sexes became almost numerically balanced. In attempting the same with Rh. marionis (p. 506) he did not succeed in affecting the ratio at all.

In the present species re-fertilisation, like ordinary fertilisation, has not been observed, and in those cases of reproduction in which males may nevertheless have taken part the sex of the offspring may not have been affected, though, curiously enough, the only males produced (besides those of the parental generation, which occur regularly among the nematodes taken while larval from the worm) have been among the offspring resulting from these cases.

The culture series made in the course of this work were for some not very apparent reason exceedingly short-lived. Had the conditions been more favourable, they could have been maintained through a much larger number of generations, and would have thrown more light on the sexual phenomena of the species.

Life-History.

(1) Investigation of Problems relating to the Life-Cycle.

It has been already shown that active and encysted larvæ of Rhabditis pellio B. infest the nephridia and the cœlomic cavity of Lumbricus terrestris respectively. It is also known that these larvæ, on the death and decay of the host in damp earth, become sexually mature and reproduce. Beyond this, except for a few scattered details mentioned in the literature of the Nematoda, the course which events take in a natural state has not been known. It was in the hope of being able to elucidate some of the interesting questions which suggested themselves in connection with the life-history that certain experiments were undertaken. The difficulties were considerable. It was found exceedingly difficult to imitate experimentally the natural conditions under which the nematode and also the worm live. Both animals were at a disadvantage, and were often unable to develop in a normal manner or in many cases to grow or even to live at all. The results obtained from this investigation are, therefore, incomplete, and still leave room for further work.

(1) The Nematode not confined to the Body of the Worm during the Latter's Lifetime.—An experiment to determine whether the larval nematode ever leaves the worm while the latter is living was several times made. A Lumb. terrestris was first thoroughly washed in order to remove any soil-nematodes which might be adhering to the outer surface of its body, and was then placed with filtered water in a watch-glass. The tail of the

worm was kept out of the watch-glass, so that no soil-nematode, which might happen to have been swallowed with the earth passing through the alimentary canal, could pass out into the water by the anal aperture. In almost every case a few larval nematodes of the active nephridial kind were found in the water around the worm after two or three hours. From the elimination of the anus as a means of exit there is every probability that the nematodes, being the active form, came from the nephridia, escaping by the nephridiopores.

That nematodes originally obtained from an earthworm will, when placed in a small quantity of water with another worm, make every effort to enter the second worm by any available orifice has been demonstrated experimentally by de Ribaucourt (**11**, p. 297) in the case of a smaller earthworm, Notogama fœtida, the brandling. I have myself on one occasion seen two larval nematodes with their anterior ends buried in the external surface of the body of a small earthworm about 1·5 inches long. They were probably entering by the pores. That the pores are capable of affording a sufficiently wide passage is shown by the effect of a sudden application of chloroform vapour. The worm is made to discharge forcibly through its pores large quantities of cœlomic fluid containing not only amœbocytes, but also occasionally both active and encysted nematode larvæ.

It seems probable, therefore, that during the life of the earthworm the larval nematodes pass out and in again by the external apertures of the cœlomic cavity and spend longer or shorter periods in the soil or even enter a different worm.

In order to ascertain by direct means whether Rh. pellio can be actually found living a free life in the soil, samples of garden earth were examined from time to time. Larval nematodes which were discovered were removed, and attempts were made to rear them to maturity with a view to establishing their identity by means of the sexual characters. From some cause not yet understood the attempts proved unsuccessful and the nematodes died before reaching maturity.

Maupas also (**10**, p. 623) has only once succeeded in finding Rh. pellio in the soil.

(2) A Period of Independent Existence passed in the Soil after the Death and Decay of the Earthworm underground.—A Lumb. terrestris was killed and allowed to decay. When the nematodes which it contained had become sexually mature and begun to reproduce, the worm was transferred to a fairly shallow glass dish, four inches in diameter, filled with soil which had previously been heated to kill off any nematodes present in it. The decaying worm was coiled up in as small a space as possible and placed in the centre of the floor of the dish. The earth was spread around and over it to a depth of about one inch and then moistened. Water was afterwards added from time to time in order to keep the earth sufficiently moist to prevent the desiccation of the nematodes. About two weeks after the experiment was begun, the soil in all parts of the dish was found to be teeming with nematodes. The worm occupied only a small space below the surface of the soil in the centre of the dish. But as far away as almost at the very edge of the soil, where it tended to become dried up, the nematodes were plentiful. Careful examination indicated that their relative abundance in different parts of the soil in the dish was no longer determined by the desire to be near to the dead worm for the sake of the food afforded by its putrefying body.

Although there were probably some thousands of nematodes in the soil, not a single adult was seen away from the worm. All those that had wandered away into the surrounding soil were larvæ, offspring of the adults introduced with the worm, and had reached the same stage of growth as that of the larvæ found in the nephridia. Six months later they were alive and plentiful but had not grown. Shortly afterwards the earth was accidentally allowed to dry and almost all of them died. But as much as eight months later, i. e. fourteen months after the beginning of the experiment, one larva was still alive and had not grown any further. I do not know whether at the commencement only one or more than one generation of offspring was produced, but the larvæ were no larger at the end of the experiment than when I first examined them shortly after the commencement.

The results of this experiment would seem to indicate that the offspring of the nematodes which mature and reproduce in a worm that dies and decays in the soil are able to wander away from their food-supply after (or even perhaps before) it is exhausted and pass a period of free existence in the earth, perhaps only until the earliest opportunity arrives of infecting a fresh worm. It appears that, before leaving the carcase of the worm, they have reached the stage of growth corresponding to that of the larvæ found in the nephridia, and while free in the soil do not under ordinary circumstances, outgrow this stage. I have shown, however, in the previous section the difficulty of finding the larvæ of Rh. pellio in the soil to prove this.

(3) No Alternate Host discovered.—Since earthworms fall a prey to moles, thrushes, blackbirds, rooks and many less highly organised animals, it was only to be expected that Rh. pellio would be met with in connection of one sort or another with them. The larval nematodes which are eaten with the worm might succumb to the action of the digestive juices or travel unharmed through the alimentary canal and out into the earth with the fæces or pass a period of their existence within the body, either remaining larval or attaining sexual maturity.

The alimentary canals of four moles were examined on different occasions. They had been feeding on earthworms amongst other things, as was evident from the presence of setæ and portions of nephridia among the contents of the stomach. Larval nematodes, both free and encysted, were found in the stomach and intestine. The cyst of one of the encysted individuals was open at the end, and the nematode inside it was seen to be alive. All the unencysted individuals were dead, except a few which appeared after two days among the rectal contents of one of the moles. All the larvæ were similar in size and appearance to the larvæ of Rh. pellio except these last, which were stouter and rather blunter-tailed. I tried to rear these and the encysted larvæ to maturity for the purpose of ascertaining whether they were Rh. pellio, but I was unsuccessful. No adult nematodes were seen. It appears likely, then, that the larvæ of Rh. pellio which are in the earthworm when the latter is swallowed by the mole succumb to the action of the digestive juices in their passage down the alimentary canal, or, if they survive, do not remain and mature but escape with the excrement into the soil.

The droppings of several thrushes were examined while still moist, and were found to contain a large number of larval nematodes similar in appearance and size to the nephridial larvæ of Rh. pellio. All were dead. But death may have been due, not to the effects of the passage through the gut, but to the frosts which prevailed at the time when the fæces were deposited. Other freshly dropped fæces of a bird have been found to contain living larval nematodes, showing that nematodes can survive a passage through the gut.

Maupas (**10**, p. 624) says that the larvæ of Rh. pellio are plentiful in all the slugs around Algiers. Whether the slug is an alternate host to the earthworm is not clear. But the impression conveyed is that the nematode can live equally well in the body of either and may pass quite casually into one or the other.

In this connection it might be mentioned that several attempts were made to rear again to maturity on decaying body-wall and on "extract" of Lumb. terrestris the larvæ which were obtained in the experiment with the worm decaying in soil in the glass dish. But all failed. This might be taken as an indication that an alternate host is necessary, but it is more likely that failure was due to other causes.

It was not discovered, therefore, whether the life-cycle of Rh. pellio is divided into two periods, one spent in the earthworm and the other in an alternate host. But it seems very probable that the mole, thrush and other animals which prey on earthworms act merely as carriers of the nematode.

(4) Transference of the Nematode from Worm to Worm within the Cocoon.—I endeavoured to discover whether the larvæ of Rh. pellio are ever transferred from the parent worm to the young worm inside the cocoon. The cocoons are often plentiful in the soil and can be procured by digging. But it is not easy to discover with certainty to which species of earthworm they belong, and I found it impossible, by digging in the earth, to obtain cocoons which I was sure belonged to Lumb. terrestris. An unsuccessful attempt was made to obtain cocoons belonging undoubtedly to Lumb. terrestris by keeping a number of mature worms of this species under natural conditions in the soil, but confined apart from all other species of earthworms.

I have, however, examined many cocoons which I found by direct search in garden soil, but without being able to ascertain to which species they belonged. They were of very varying sizes and belonged no doubt to worms of more than one species. Nematodes were found living within several of them in the albuminous fluid bathing the embryo worm. The nematodes were larval and resembled the nephridial form of Rh. pellio. I attempted to rear them to maturity in order to determine their identity, but was unsuccessful.

I also removed all the young worms that were ready to hatch from the cocoons, killed them and allowed them to decay in a little water. Had they been already infected while in the cocoon, the nematodes would have appeared when they decayed. But this did not happen.

More evidence is required on this question. Neither the identity of the cocoons examined nor of the nematodes found inside them was known. But it appears quite probable that the larvæ of Rh. pellio do not infect the embryonic Lumb. terrestris in the cocoon even though they may be present with it.

If the larval nematode found in the cocoon be Rh. pellio it is not difficult to understand how it gets there. When the cocoon is being slipped forward towards the head of the worm it must pass over the nephridiopores, and it is quite easy to imagine a few nematodes escaping from the nephridia into the cocoon through these apertures.

(5) Occasions when the Larval Nematode attains Sexual Maturity.—The sexually mature adult of Rhabditis pellio is found engaged in reproduction in large numbers in the dead earthworm decaying in soil, as is shown in the case of worms which have died a natural death in the earth and are found there in a state of putrefaction, as well as by artificial methods. But I have never seen the adult or its eggs or newly hatched larvæ in the live earthworm, and I do not believe that they ever occur there.

Thinking that Rh. pellio might possibly attain the mature condition in the soil as well as in the dead worm, I have searched samples of earth for it. But I have failed to find it.

It is not, therefore, certain whether the larval Rh. pellio in the course of its development becomes sexually mature in ordinary soil or only in the dead worm. But if a larva which has passed out into the soil from the body of a live worm finds a quantity of nutritive substance such as the decaying carcase of some animal, it seems reasonable to suppose that under such circumstances it will mature and reproduce just as it would have done had it remained in the worm till the latter died. Such behaviour would correspond to that of the larvæ of the free-living soil-inhabiting species, which grow into adults and propagate when they find some putrefying substance in the soil.

(6) Mode of Infection of a Fresh Worm by the Larval Nematode.—From the fact that the adult nematode is entirely absent from the live earthworm it is evident that the infection of a fresh worm is carried out by the larva.

There are two possible ways of entrance into the body: (i) through the external apertures of the cœlomic cavity, or (ii) by the mouth or anus into the gut and through the gut-wall.

(i) I have not cut sections of the gut of Lumb. terrestris to ascertain whether nematodes can be seen actually traversing the gut-wall, and I have no evidence on this question except the fact that on three occasions I have found species of larval nematodes alive among the soil contents of the gut. Whether the nematode is capable of making its way through the tissues of the gut-wall is not quite certain. The genus Rhabditis is not provided, like some genera, with piercing mouth-parts, nor do the nematodes in a nephridium removed from a freshly killed worm appear at all capable of migrating through even its relatively thin wall. At the same time they must be able, to some extent, to push their way in among the tissues of the worm to be found imbedded in the muscular layer of the body-wall, as is sometimes the case.

(ii) With regard to the other way of entrance, de Ribaucourt, as already shown (**11**, p. 297), has contributed definite evidence that nematodes can enter a worm by the cœlomic pores. I have on one occasion seen larval nematodes apparently entering by the pores, and I have shown that the larvæ can leave the worm by the same means. K. C. Schneider (**13**, p. 423) believes that the nematodes in the bladder of the nephridium have wandered in through the nephridiopore.

It seems probable, therefore, that whether or not Rh. pellio in the natural state infects the worm through the gut-wall as well, it certainly does so by entering through the dorsal pores, nephridiopores or reproductive apertures, or possibly by all of these.

(7) Reason for the Presence of Rh. pellio in the Worm in Two Different Larval Conditions.—The most likely reason for the existence of two kinds of larvæ—free and active in the nephridia and seminal vesicles, encysted and quiescent in the cœlomic cavity—accords with the supposition that the nematodes enter the worm by the pores.

(i) Firstly, those which pass in by the nephridiopores find themselves, as K. C. Schneider says, in the bladder-parts of the nephridia where they remain as the nephridial form.

Those, too, which find their way in by the spermiducal apertures and travel up the vasa deferentia are the same as are found on opening the seminal vesicles.

(ii) Secondly, those which enter by the dorsal pores and the oviducal apertures find themselves in the cœlomic cavity. Here they are attacked as foreign bodies by the amœboytes and encyst. Keng (8, p. 391) describes the way in which the amœbocytes surround and cover nematodes in the cœlom, hampering their movements by means of fine protoplasmic threads into which they can become drawn out. He gives a drawing (pl. v, fig. 44) of a nematode struggling with amœbocytes. When the nematode is completely covered it apparently sheds the outer layer of its cuticle. The amœbocytes soon die and turn brown. The cyst is composed of the loosened outer layer of cuticle with its investment of dead brown amœbocytes. The completely and partially encysted nematodes are gradually worked backwards through successive segments by the movements of the worm until they reach the tail, where, with cysts of Monocystis and discarded setæ, they are compacted by pressure into the flattened oval masses and cemented together by their investment of broken-down cœlomic corpuscles. The great majority of the nematodes found in the cœlomic cavity of a fresh-killed worm are encysted. But in addition to these there are some which are covered with amœbocytes but not completely enclosed by a cyst, and some which are quite free. Those that are found quite free have probably not yet been attacked by amœbocytes. Those that are covered with amœbocytes but are not fully encysted were probably about to become so when the worm was killed. The latter, being only slightly encumbered, are no doubt those which, disengaging themselves from the imprisoning lymph-cells, are the first to be seen escaping from the brown bodies. The only occasion that I know when the larvæ emerge from their cysts is on the death and decay of the worm, when the food supply becomes plentiful and nutritious. But only a certain proportion of the fully encysted larvæ do so. The remainder appear to have degenerated, for they do not recover from the conditions of diminished vitality under which they have been existing. Those which emerge are therefore the more recently encysted ones, which have not yet begun to degenerate.

(8) Nature of Food.—Rhabditis pellio belongs to a genus, most of whose members are free-living in the soil and mature and reproduce on animal or vegetable substances in putrefaction. It resembles these in the conditions under which it matures and reproduces. But it differs from them in spending all or part of its larval period in the body-cavity of an animal instead of in the soil. It does not appear to do any damage to the worm. It occurs in the largest and healthiest-looking individuals in quite as large numbers as in weakly specimens. Moreover, having, like all the species of the genus Rhabditis, an unarmed buccal cavity, it is incapable of feeding on the tissues of the living worm.

Its food consists of liquids, which are taken in by the suctorial action of the œsophagus. It grows most rapidly in media which are swarming with bacteria, which shows that it is upon the bacteria or the products of their action that it lives. What Potts says of the free-living species of the genera Rhabditis and Diplogaster applies to this species also. He says (**15**, p. 444): "It is only in the presence of great numbers of bacteria, or the substances formed by them, that the nematodes thrive so well. . . . It has not been discovered whether digestion takes place by the secretion of juices dissolving the protoplasm of the bacteria, or is merely confined to the absorption of soluble substances present in the culture fluid and prepared by the action of bacteria. . . . An easily observable phenomenon of nematodes in culture is the rapid pumping action of the second œsophageal bulb and the rectum, and it may be argued from this that the nutriment obtained from the stream of fluid so constantly passing through the alimentary canal is in the form of easily abstracted soluble substances. The insignificant development of glandular cells (which are found only in the œsophagus) may be cited against an intra-intestinal digestion of the bacteria, and, whatever else its significance may be, the chitinous layer which lines the alimentary canal throughout must prevent an ingestion of bacteria by the endoderm cells themselves in such a way as Colpidium preys upon the bacteria of the soil."

The active nephridial larvæ feed on the bacteria or bacterial products which pass down the nephridial tube in the current of cœlomic fluid, or which may congregate in the dilated bladder-part. But the number of bacteria in the living worm must be relatively small compared with the number produced on its decay, and there is, therefore, little or no growth in this condition. Hence the larval period is a long one. But on the death and decay of the worm large numbers of bacteria are produced, and, food now being plentiful, growth is rapid, and the nematodes mature and reproduce in a few days.

Rhabditis pellio, then, not only belongs to a genus most of whose species are free-living, but in all respects except its habitat appears to behave like one of the free-living species of that genus. The rôle which Maupas (**10**, p. 623) ascribes to it when he speaks of it as "locataire inoffensif" is the true one, and the advantages which it gains from its association with the worm are protection and dissemination.

(2) Probable Course of the Life-Cycle.

The results obtained in the foregoing investigation are not conclusive, but they suggest, I think, that the life-history of Rhabditis pellio is somewhat as follows:

When an infested Lumbricus terrestris dies in moist earth, the larvæ of Rh. pellio which it contains feed on the nutriment afforded by its putrefying carcase, the encysted individuals emerging from their cysts. Growth is rapid. Within a week they develop into sexually mature males and females, and the latter reproduce. Reproduction is often—possibly always—hermaphrodite. But sometimes it seems to be bisexual. I am not certain whether in a natural state the body of the decaying worm is capable of affording sufficient nutriment for the production by these offspring, in their turn, of a further generation. The young reach the same stage of growth as that of the nephridial larvæ. They then leave the carcase of the worm and wander into the soil. Here they live for a longer or shorter period but do not increase in size. Sooner or later they infect an earthworm, making their way in by the external apertures of the cœlom. Those that enter by the nephridiopores take up their position in the terminal, bladder-like part of the nephridia. Those that use the spermiducal apertures travel up the vasa deferentia and occupy the seminal vesicles. Lastly, those that pass in by the dorsal pores and the oviducal apertures find themselves in the cœlom, where, being attacked by the amœbocytes, they encyst. These encysted larvæ coated with amœbocytes are worked backwards by the movements of the worm till they come to rest in the tail end of the worm, where, together with other foreign bodies, such as cysts of Monocystis and discarded setæ, and with masses of dead brown-coloured amœbocytes, they are compressed and cemented into the brown bodies which are found there.

In this larval condition the nematodes remain during a protracted period without growth, the encysted form without movement, until on the death and decay of the worm in the soil they grow, mature and reproduce.

When infested worms are eaten by moles, thrushes or other predatory animals, it is probable that the nematodes travel down the alimentary canal, and, whether alive or dead, pass out with the fæces into the soil. I have not yet any evidence of the existence of an alternate host, within whose body the nematode spends a part of its life-history.

During its larval existence the active nephridial form probably passes out and in by the external apertures of the cœlom and spends longer or shorter periods in the soil. Or it may change hosts, entering and inhabiting another worm. But if, while in the soil, it finds some animal or vegetable substance in putrefaction, it may mature and reproduce on the spot, as it would have done in the worm had it remained till the death of the latter.

This passing backwards and forwards between the body of the worm and the soil may have interesting consequences when it takes place during the detachment of the cocoon of a worm engaged in reproduction. The finding of larval nematodes within the closed cocoon suggests that, when the latter is being slipped forwards towards the head, some of the nematodes in the nephridia pass out into the cocoon through the nephridiopores. But the young worm in the cocoon has not been found to be infected, and the inference is that the presence of the nematode is only accidental, and that, when the young worm hatches out of the cocoon, the nematode escapes into the soil.

Summary of Principal Results.

(1) The active larval nematodes inhabiting the nephridia of the common earthworm, Lumbricus terrestris Linn., and the encysted larvæ found in the cœlom of the same host belong to the same species of Rhabditis.

(2) This species I regard as distinct from Rhabditis pellio Schneider, with which it has hitherto been confused.

Bütschli first described it but regarded it as merely a form of Rh. pellio. Rh. pellio is, however, a soil species which may mature on decaying earthworms; the species described in this paper is a parasite of the earthworm and has not hitherto been recorded with certainty in the soil. I propose to designate it provisionally by the name Rhabditis pellio Bütschli, non Schneider.

(3) The dimensions attained by the adults of this species vary considerably according to the nutritive value of the culture medium employed. Decaying earthworm, the food material on which the species grows to sexual maturity in a natural state, has been found more nutritious than the usual artificial media, such as peptone, and promotes correspondingly greater growth.

(4) A medium consisting of extract of earthworms has

been devised, by which the natural food is conveyed in a nutritious form, convenient for cultural purposes.

(5) The nematodes removed from the freshly killed worm and reared in cultures, or obtained from the dead worm decaying under natural conditions, develop into males and females. Subsequent generations, however, bred under cultural conditions, consist in almost all cases of females only.

(6) Examination of these cultures, consisting of females only, reveals the fact that they are in reality hermaphrodite.

(7) Reproduction is frequently—perhaps always—hermaphroditic. But cases occur in which it appears to be bisexual —that is to say, both hermaphrodites and true females may exist side by side in the same species, as in Rhabditis marionis Maupas.

No "partial hermaphrodites" have been found, nor have any cases of "re-fertilisation" been observed.

(8) The numerical ratio of males to females is extraordinarily variable, and no rule governing the fluctuations has yet been found.

(9) I have not been able to follow out the life-history in its entirety, but evidence is afforded of the probable mode of transmission and of infection.

BIRMINGHAM,
November 26th, 1912.

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EXPLANATION OF PLATE 37.

Illustrating Mr. G. E. Johnson's memoir "On the Nematodes of the Common Earthworm."

acc. p. Accessory piece. *amœb.* Envelope of dead amœbocytes. *buc. cav.* Buccal cavity. *burs.* Bursa with bursal papillæ. *cy.* Cyst. *ex. can.* Excretory canal. *ex. po.* Excretory pore. *gen. rud.* Genital rudiment. *int.* Intestine. *nerv. col.* Nerve-collar. *œs. b.* Œsophageal bulb. *ov.* Ovary with developing eggs. *ovd.* Oviduct or "seminal receptacle" with spermatozoa. *pap.* Papilla. *rect.* Rectum. *rect. gl.* Rectal glands. *sp.* Copulatory spicules. *test.* Testis with developing spermatozoa. *ut.* Uterus with fertilised eggs. *v. d.* Vas deferens. *vulv.* Vulva.

Fig. 1.—A mature male of Rhabditis pellio in latero-ventral view, showing the reproductive organs. (× 90.)

Fig. 2.—A mature hermaphrodite of Rh. pellio in lateral view, showing the reproductive organs. This is the typical form developed in, and nourished on, the decaying earthworm. (× 90.)

Fig. 3.—A mature hermaphrodite of Rh. pellio fed on peptone, showing its inferiority in size to the typical hermaphrodite nourished on the decaying earthworm, and the smaller number of eggs in the uterus. (× 90.)

Fig. 4.—An active larval individual of Rh. pellio from the nephridium, showing the genital rudiment. (× 230.)

Fig. 5.—An encysted larval individual of Rh. pellio from the cœlom in the act of escaping from the cyst. (× 230.)

Fig. 6.—The anterior end of a hermaphrodite of Rh. pellio in lateral view, showing the mouth-parts, nerve-collar and excretory system. (× 350.)

Fig. 7.—The tail of a hermaphrodite of Rh. pellio in ventral view showing the rectal glands and caudal papillæ. (× 350.)

Fig. 8.—The tail of a hermaphrodite of Rh. pellio in lateral view, showing the short rectum in distension and the anus. (× 350.)

Fig. 9.—Tail of a mature male of Rh. pellio in latero-ventral view, showing the bursa, bursal papillæ and copulatory spicules. (× 350.)

Fig. 10.—Tail of a male of Rh. pellio in dorso-lateral view showing the copulatory spicules and the accessory piece. (× 350.)